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Lab Resource: Single Cell Line

Generation of human induced pluripotent stem cell line UNIGEi001-A from a 2-years old patient with Mucopolysaccharidosis type IH disease

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A B S T R A C T

Mucopolysaccharidosis type I-Hurler (MPS1-H) is the most severe form of inherited metabolic diseases caused by mutations in the *IDUA* gene. The resulting deficiency of alpha L-iduronidase enzyme leads to a progressive accumulation of glycosaminoglycans in lysosomes which damages multiple organs and highly reduces life expectancy of affected children. Skin fibroblasts of a 2-year-old MPS1-H male, carrying two mutations in each IDUA alleles (H358_T364del; W402X), were reprogrammed into induced pluripotent stem cells (iPSCs) using the CytoTune-iPS Sendai Reprogramming method applying Yamanaka-factors (*OCT4, SOX2, KLF4, c-MYC*). iPSCs expressed pluripotency transcription factors while iPSC-derived embryoid bodies reveal markers of the three germ layers.

Resource Table:

Unique stem cell line identifier	UNIGEi001-A
Alternative name(s) of stem cell line	MPS1-H.2y.S4
Institution	University of Geneva (UNIGE), Geneva, Switzerland
Contact information of distributor	Karl-Heinz Krause; Karl-Heinz.Krause@unige.ch
Type of cell line	iPSC
Origin	human
Additional origin info	Age: 2 Sex: male Ethnicity: Caucasian
Cell Source	Skin fibroblasts
Clonality	Clonal
Method of reprogramming	Sendai virus
Genetic modification	Yes
Type of modification	Hereditary
Associated disease	Mucopolysaccharidosis type I (MPS1), also named Hurler syndrome
Gene/locus	Two different mutations were identified. One in each IDUA single allele (Cytogenetic location: 4p16.3) - In exon 8: NM_000203.5(IDUA):c.1073_1093del (p.His358_Thr364del) - In exon 9: NM_000203.4(IDUA):c.1205 G>A (p.Trp402Ter)
Method of modification	Not applicable
Name of transgene or resistance	Not applicable
Inducible/constitutive system	Not applicable
Date archived/stock date	July 2014
Cell line repository/bank	https://hpscreg.eu/cell-line/UNIGEi001-A
Ethical approval	Parents of patient informed consent obtained. Ethical approval was obtained from the competent authority to establish and maintain human iPSC lines (in Hungary: Medical Research Council (in Hungarian: Egészségügyi Tudományos Tanács – Tudományos és Kutatásetikai Bizottság; ETT-TUKEB) ETT-TUKEB 834/PI/09, 8-333/2009-1018EKU);

1. Resource utility

Mucopolysaccharidosis type I (MPS1), or Hurler syndrome, is a rare and severe lysosomal storage disorder progressively affecting many organs and leading to death during childhood if treatments are delayed. Induced pluripotent stem cells, generated from MPS1 patient may represent a valuable resource for modelling the disease and deciphering pathological processes.

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Table. 1
Characterization and validation.

Classification	Test	Result	Data
Morphology	Microscopic observation Phase contrast images	Normal	Fig. 1C
Phenotype	Qualitative analysis Immunocytochemistry	Positive for OCT4, NANOG, SOX2	Fig. 1F
	Quantitative analysis Flow cytometry	Expression of *OCT4, NANOG* and *SOX2* (>94%)	Fig. 1E
Genotype	CGH array	Normal male karyotype: arr(1–22)x2,(XY)x1 Analytic resolution 200 kb No pathogenic or non-pathogenic copy number variant detected	Fig. 1B
Identity	Microsatellite PCR (mPCR) OR STR analysis	N/A 6 loci tested, all matched	N/A Available with the authors
Mutation analysis (IF APPLICABLE)	Sequencing	Two different mutations were identified. They are distributed on each *IDUA* allele (Cytogenetic location: 4p16.3): - In exon 8: NM_000203.5(IDUA):c.1073_1093del (p.His358_Thr364del) - In exon 9: NM_000203.4(IDUA):c.1205 *G>A* (p.Trp402Ter)	Fig. 1A
	Southern Blot OR WGS	N/A	N/A
Microbiology and virology	Mycoplasma	PCR-based Mycoplasma test	Supplementary Fig. 1
Differentiation potential	Embryoid body formation Phase contrast images	Normal morphology of Embryoid bodies	Fig. 1H
	PCR	Negative expression Pluripotent marker Positive expression of specific markers of the three germ layers	Fig. 1G
	Spontaneous *in vitro* differentiation into the three germ layers	*In vitro* differentiation into derivatives of the three germ layers: endoderm, α-fetoprotein (AFP); mesoderm, Forkhead Box A2 (FOXA2); and ectoderm, β3-tubulin (TUBB3)	Fig. 1I
Donor screening (OPTIONAL)	HIV 1 + 2 Hepatitis B, Hepatitis C	N/A	N/A
Genotype additional info (OPTIONAL)	Blood group genotyping	N/A	N/A
	HLA tissue typing	N/A	N/A

2. Resource details

Mucopolysaccharidosis type I-Hurler (MPS1-H), the most severe form of MPSs, is a rare lysosomal storage disorder caused by the deficiency of alpha L-iduronidase (IDUA), which is the needed enzyme to catabolize glycosaminoglycans (GAGs) (Giugliani et al., 2010). Impairment of its activity leads to progressive accumulation of GAGs followed by dysfunction of many biological systems and organs, such as affections of cardiovascular, respiratory and central nervous system. Without enzyme replacement therapy or hematopoietic stem cell transplantation, this autosomal recessive disorder is fatal in early childhood (before 5 years old) (Tolar et al., 2008).

Skin fibroblasts were isolated from skin tissues of a 2-year-old male, MPS1-H diagnosed by Division of Metabolism and Children's Research Center of University Children's Hospital of Zürich (Switzerland). Loss-of-function of alpha L-iduronidase and resulting MPS1-H symptoms have genetically been associated to the presence of two distinct mutations distributed on the two alleles of the *IDUA* gene (NM_000203.5(IDUA):c.1073_1093del (p.His358_Thr364del) and NM_000203.4(IDUA):c.1205 G>A (p.Trp402Ter)) (Table 1, Fig. 1A).

MPS1 fibroblasts have been reprogrammed into MPS1 iPSC line by using integration-free CytoTune-iPS Sendai Reprogramming system, using the Yamanaka factors (*OCT4, SOX2, KLF4*, and *c-MYC*) (Takahashi et al., 2007). After few passages, newly generated MPS1-H.2y.S4 iPSC were fully characterized. First, the genetic identity of isolated iPSC and the parental MPS1 patient fibroblasts have been confirmed by STR analysis (Table 1). Both *IDUA* mutations, identified in fibroblasts, were also detected in *resulting iPSC* (Fig. 1A). At passage 12, normal karyotype and genome integrity were assessed by Comparative Genomic Hybridization (*CGH*) array. While this technique cannot identify balanced translocations, cells present a normal karyotype without genomic imbalances (Fig. 1B). Performance of a PCR-based Mycoplasma test certified our cell culture as mycoplasma-free (Fig. S1).

Based on the clear borders of colonies that are composed of small, tightly packed cells with large nucleoli, these cells display morphological features and organization of pluripotent stem cells (Fig. 1C). Although alkaline phosphatase activity was detected in the colonies (Fig. 1D), validation of the reprogramming of fibroblasts into *bona fide* iPSC was also confirmed by the expression of pluripotency transcription factors by flow cytometric analysis (Fig. 1E) and immunofluorescence staining (Fig. 1F). Indeed, more than 94% of cells were positive for NANOG, OCT4 and SOX2 by FACS analysis.

In order to further confirm their pluripotency, embryoid bodies (EBs) have been generated from the cells (Fig. 1H) and were spontaneously differentiated *in vitro* for 24 days. Differentiation potential of the cells was validated by the expression of specific markers of the three germ layers, both by immunofluorescence staining (Fig. 1I) and PCR (Fig. 1). Expression of *SOX17* and AFP revealed a differentiation toward the endoderm, while *ACTA2* and FOXA2 highlighted a mesodermal pattern and finally TUBB3 and *MAP1B* the presence of ectoderm germ layer.

In conclusion, our newly reprogrammed MPS1-H.2y.S4 iPSC presents all features of pluripotent stem cells and is ready to be used for MPS1-H modelling or for drug discovery studies.

3. Materials and methods

3.1. Human dermal fibroblast reprogramming

Fibroblasts, isolated from the skin biopsy of a 2-year-old male MPS1 patient, were expanded in fibroblast medium (DMEM, 10% fetal bovine serum (FBS), 1% Non-Essential amino acids (NEAA), 1% Sodium pyruvate, 1% Glutamine and 1% Pen/Strep). An amount of 2×10^5 MPS1 fibroblasts were plated on 6-well plate and transduced with the Yamanaka factors (*OCT3/4, NANOG, KLF4, c-MYC*), using CytoTune®-iPS Sendai Reprogramming Kit (Thermo Fisher Scientific) at an MOI of 5:5:3. Cells were maintained in fibroblast medium until D4, when medium was switched to mTeSR™1 medium (StemCell Technologies). Colonies were picked manually between D19 and D24 on Matrigel®-coated culture dishes and were routinely passaged every 5–6 days, at a split ratio of 1:5, using 0.5 mM EDTA (Sigma-Aldrich). All cells were cultured at 37 °C in humidified atmosphere containing 5% CO_2.

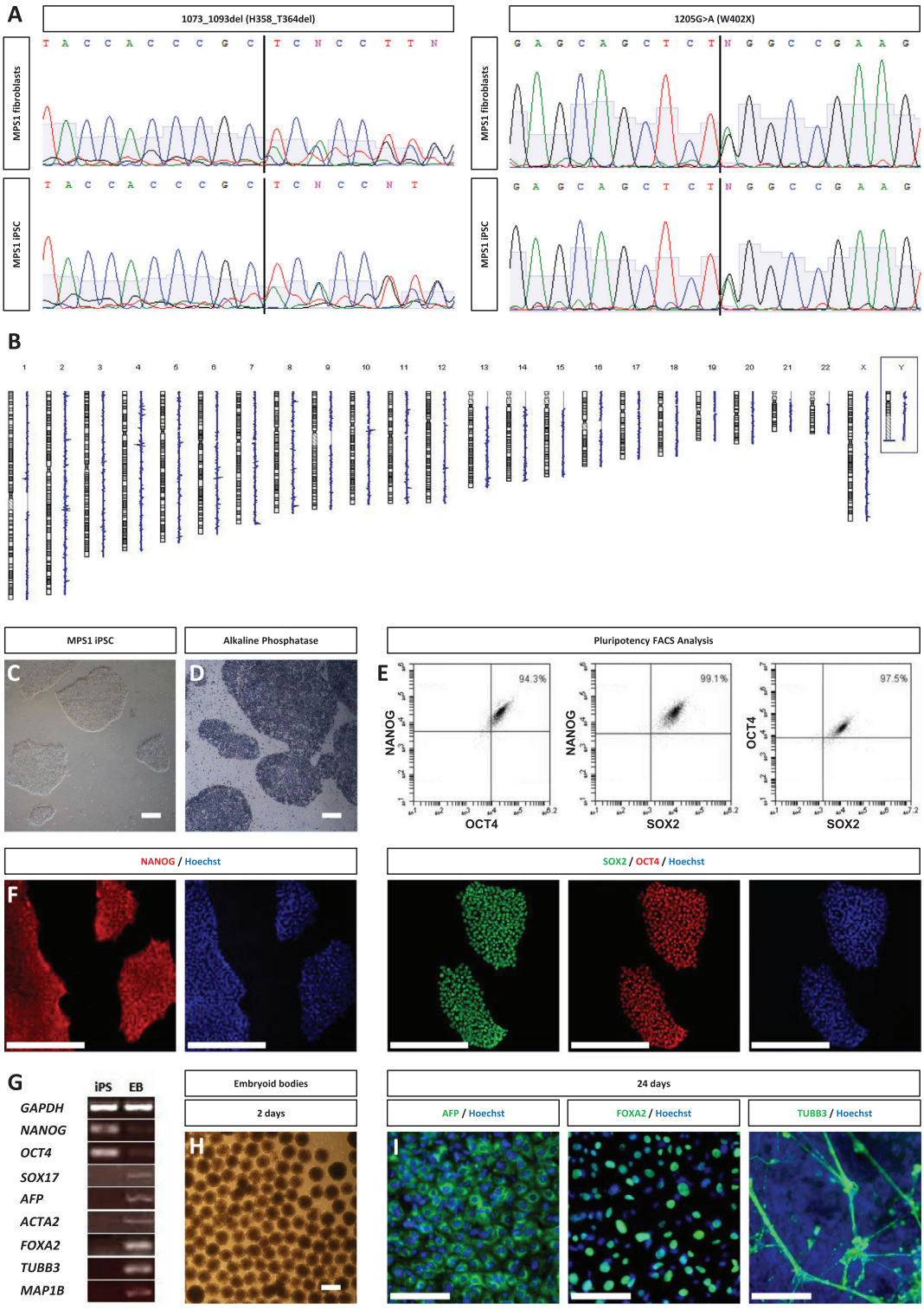

Fig. 1. Characterization of MPS1-H.2y.S4 iPSC line. (A) Sanger sequencing of *IDUA* regions revealing two distinct mutations associated to MPS1-H in parental MPS1 patient fibroblasts and in the newly established MPS1 iPSC line. (B) CGH array of MPS1 iPSC line showed normal karyotype of a male subject. (C) Generated MPS1 iPSC line cells displayed morphological features of pluripotent stem cells and were (D) alkaline phosphatase positive. (E) FACS analysis and (F) immunofluorescence staining revealed the presence of high levels of the pluripotent markers NANOG, OCT4 and SOX2. Nuclei was counterstained with Hoechst. (G) PCR analysis confirmed the expression of pluripotent transcription factors *NANOG* and *OCT4* in iPSC and the expression of specific markers of endoderm (*SOX17* and *AFP*), mesoderm (*ACTA2* and *FOXA2*), and ectoderm (*TUBB3* and *MAP1B*) into iPSC-derived embryoid bodies. *GAPDH* was used as housekeeping gene. (H) Floating embryoid bodies 2 days after aggregation. (I) Differentiation potential of MPS1 iPSC line was confirmed by immunofluorescence staining of spontaneously differentiated embryoid bodies into the three germ layers at 24 days: endoderm (AFP), mesoderm (FOXA2), and ectoderm (TUBB3). Nuclei was counterstained with Hoechst. All scale bars = 50 μm.

3.2. Pluripotency flow cytometric assay

Expression of pluripotent stem cell transcription factors of MPS1-H.2y.S4 iPSC was validated by flow cytometry, using BD Stemflow Human Pluripotent Stem Cell Transcription Factor Analysis Kit® (BD Biosciences) according to the manufacturer's instructions. Acquisition was performed using ACCURI 6 flow cytometer. Data analysis was carried out with BD Accuri C6 software.

3.3. Alkaline phosphatase staining

iPSC colonies were fixed with 4% paraformaldehyde (PFA) for 10 min at room temperature (RT), then incubated with NBT solution (Sigma-Aldrich) for 30 min at RT. Stained colonies were observed under Eclipse Ts2 inverted microscope (Nikon). Scale bar = 50 µm.

3.4. Embryoid bodies assay

The iPSC clones were spontaneously differentiated into the three embryonic germ layers. Colonies were dissociated with Accutase® for 5 min at 37 °C and uniform EBs were formed by plating 1000 cells/microwell of *AggreWell*™ plates, in mTeSR™1 medium supplemented with 10 µM of Rock inhibitor (Abcam Biochemicals). One day after, EB were collected and cultured in rotation (60 rpm, orbital shaker) for 5 days in DMEM supplemented with 20% FBS, 1% NEAA, 1% Glutamine, 1% Pen/Strep and 0.1 mM β-mercaptoethanol. On D6, EBs were transferred to gelatin-coated plates and maintained in same medium for additional 19 days. Medium was changed every 2–3 days.

3.5. Immunocytochemistry

Following 4% PFA fixation for 10 min at RT, iPSC colonies or embryoid bodies were incubated 30 min at RT in blocking solution constituted by PBS containing 0.1% Triton X-100 and 3% BSA, to block nonspecific binding. Cells were then incubated 1 hour at RT, with the specific primary antibodies respectively (Table 2), diluted in the blocking solution. Appropriate secondary antibodies were incubated for 45 min at RT. Nuclei were stained with DAPI. Images were captured with a LSM-700 (Zeiss) confocal microscope. Scale bar = 50 µm.

3.6. RNA extraction and RT-PCR

RNA was extracted by using RNeasy Mini kit (Qiagen) and reverse transcription was performed using the PrimeScript RT Reagent Kit (Takara) according to the manufacturer's instructions. PCR amplification was performed with a T3 Thermocycler PCR system (Biometra), by using Taq DNA Polymerase (Qiagen). The temperature cycling protocol was set as follows: initial degeneration step, 3 min at 95 °C; followed by 35 cycles of denaturation: 30 s at 95 °C; annealing: 30 s at 60 °C; extension, 45 s at 72 °C; final holding, 10 min at 72 °C. Primers are listed in Table 2.

3.7. Identification of MPS1 mutations

Genomic DNA was isolated from MPS1 fibroblasts and MPS1 iPSC by using the DNeasy® Blood & Tissue kit (Qiagen). Genotyping primers localized on intron 7 and exon 9 (Table 2) were used to amplify by PCR, the region of IDUA gene containing both mutations. PCR amplification was performed with a T3 Thermocycler PCR system (Biometra), by using Q5® High-Fidelity DNA Polymerase (New England Biolabs). The thermal cycling was performed as follows: initial degeneration step, 1 min at 95 °C; 35 cycles (1 min at 95 °C; 1 min at 58 °C; 1 min at 72 °C); and a final holding, 10 min at 72 °C. PCR products were purified on 1% agarose gel before sequencing. To determine the allelic distribution of the 2 mutations further sequencing of TOPO cloned single PCR-fragments was performed.

3.8. CGH array

Oligonucleotide array-CGH was performed with the Human CGH Microarray Kit 4 × 44 K (Agilent Technologies). Data analysis was carried out with the Agilent Genomic Workbench software v6.5. Results

Table. 2
Reagents details.

Antibodies used for immunocytochemistry/flow-cytometry			
	Antibody	Dilution	Company Cat # and RRID
Pluripotency marker (IF)	Rabbit anti-OCT4	1:100	abcam, Cat# ab18976, *RRID:* AB_444,714
Pluripotency marker (IF)	Mouse anti-SOX2_[20G5]	1:200	abcam, Cat# ab171380, *RRID:* AB_2,732,072
Pluripotency marker (IF)	Goat anti-NANOG	1:100	Thermo Fisher Scientific, Cat# PA5-18,406, *RRID:* AB_10,985,008
Endodermal marker (IF)	Mouse anti-AFP	1:250	Santa Cruz, Cat# *Sc*-8399, RRID: AB_626,665
Mesodermal marker (IF)	Mouse anti-FOXA2	1:100	Santa Cruz, Cat# *Sc*-101,060, RRID: AB_1,124,660
Ectodermal marker (IF)	Mouse anti-β3-tubulin (TUBB3)	1:200	Sigma-Aldrich, Cat# T8660, RRID: AB_477,590
Primers			
	Target	Forward/Reverse primer (5′ − 3′)	
Pluripotency marker (PCR)	*NANOG* (138 bp)	GATTTGTGGGCCTGAAGAAA/ TTGGGACTGGTGGAAGAATC	
Pluripotency marker (PCR)	*OCT4* (81 bp)	AGTGCCCGAAACCCACACTG / ACCACACTCGGACCACATCCT	
Housekeeping gene (PCR)	*GAPDH* (226 bp)	GAAGGTGAAGGTCGGAGTC / GAAGATGGTGATGGGATTTC	
Targeted mutation sequencing	*IDUA*_int.7_Fwd (683 bp)	CCACCTTCCTCCCGAGAC	
	*IDUA*_ex.9_Rev	GTCGTCGCTCGCGTAGAT	
Endodermal marker (PCR)	*SOX17* (374 bp)	GTGAATCTCCCCGACAGC/ TGTTTTGGGACACATTCAAAGC	
Endodermal marker (PCR)	*AFP* (301 bp)	AGAGTTGCTAAAGGATACCAGGA/ AGGCCAATAGTTTGTCCTCAC	
Mesodermal marker (PCR)	*ACTA2* (228 bp)	AATACTCTGTCTGGATCGGTGGCT / ACGAGTCAGAGCTTTGGCTAGGAA	
Mesodermal marker (PCR)	*FOXA2* (98 bp)	TGCACTCGGCTTCCAGTATG/ GGAGGAGTAGCCCTCGG	
Ectodermal marker (PCR)	*TUBB3* (103 bp)	GGCCTGACAATTTCATCTTTGG/ ACCACATCCAGGACCGAATC	
Ectodermal marker (PCR)	*MAP1B* (87 bp)	CTGTGGAAAAGGCAGCAAAAC/ CAGCATTCTTGGTCTCCTTGTCT	
PCR-based mycoplasma	mycoplasma (500 bp)	GGCGAATGGGTGAGTAACACG/ CGGATAACGCTTGCGACCTAT	
	ribosomal protein L32 (housekeeping gene) (350 bp)	GTGAAGCCCAAGATCGTCAA/ TTGGTGACTCTGATGGCCAG	
Short tandem repeat (STR) analysis	*D4S2364*	[FAM]-CTAGGAGATCATGTGGGTATGATT / GCAGTGAATAAATGAACGAATGGA	
	D2S441	[VIC]-CTGTGGCTCATCTATGAAAACTT/ GAAGTGGCTGTGGTGTTATGAT	
	D1S1677	[VIC]-TTCTGTTGGTATAGAGCAGTGTTT/ GTGACAGGAAGGACGGAATG	
	D10S1248	[FAM]-TTAATGAATTGAACAAATGAGTGAG/ GCAACTCTGGTTGTATTGTCTTCAT	
	D14S1434	[VIC]-TGTAATAACTCTACGACTGTCTGTCTG/ GAATAGGAGGTGGATGGATGG	
	D22S1045	[VIC]-ATTTTCCCCGATGATAGTAGTCT/ GCGAATGTATGATTGGCAATATTTTT	

are given in GRCh37/hg19 genome version.

3.9. PCR-based mycoplasma test

20 000 cells were collected during passage. Cells have been lysed with 200 µg/ml of proteinase K in DirectPCR Lysis Reagent (Viagen Biotech) for 1 h at 56 °C and 45 min at 85 °C. Two PCR were performed from resulting lysate with specific primers of mycoplasma and ribosomal protein L32, listed in Table 2.

3.10. Short tandem repeat (STR) analysis

PCR amplification of STR loci was achieved as described in Coble et al. (Coble M.D, Butler J.M. *J Forensic Sci* 2005). The allelic ladders were separated electrophoretically using size Standard GeneScan LIZ500 (Applied Biosystems). Sequencing was analyzed using Applied Biosystems 3730XL DNA Analyzer. with the following conditions: injection time, 10 s; injection voltage, 1.6 kV; run time, 2100s; run voltage, 15 kV; capillary length, 50 cm; polymer, POP7; filter, Dye Set G5. The primer sequence are listed in Table 2.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.scr.2019.101604.

References

Giugliani, R., Federhen, A., Rojas, M.V., et al., 2010. Mucopolysaccharidosis I, II, and VI: brief review and guidelines for treatment. Genet. Mol. Biol. 33, 589–604.
Takahashi, K., Tanabe, K., Ohnuki, M., et al., 2007. Induction of pluripotent stem cells from adult human fibroblasts by defined factors. Cell 131, 861–872.
Tolar, J., Grewal, S.S., Bjoraker, K.J., et al., 2008. Combination of enzyme replacement and hematopoietic stem cell transplantation as therapy for Hurler syndrome. Bone Marrow Transplant 41, 531–535.